

REMARKS

Claims 52-63 are pending in the present application and at issue. It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Rejection of Claims 35-40 and 42-51 under 35 U.S.C. 103

Claims 35-40 and 42-51 are rejected under 35 U.S.C. 103 as being unpatentable over Veit et al. (U.S. Patent No. 7,244,597 or WO 02/38787), Svendsen et al. (WO 96/23874), and Nagasaka et al. (*Appl. Microbiol. Biotechnol.* 50: 323-330 (1998)). This rejection is respectfully traversed.

Veit et al. disclose a process of producing ethanol comprising primary and secondary liquefaction steps, wherein the primary liquefaction is performed at a temperature of 60-95°C and the secondary liquefaction step is performed at a temperature of 95-140°C to further gelatinize the starch.

However, Veit et al. do not teach or suggest a process of producing ethanol, comprising saccharification of raw starch (*i.e.*, ungelatinized starch).

The Office states that "The difference between the reference of Veit et al. and the instant invention is that the reference of Veit et al. does not use a glucoamylase having at least 98-99% sequence identity to SEQ ID NO: 2, wherein the sugar concentration is kept at a level below 3 wt % nor a hybrid alpha-amylase comprising an *Aspergillus niger* acid alpha-amylase catalytic domain (CD) and a carbohydrate-binding module (CBM) from *Aspergillus kawachi* alpha-amylase." This is respectfully traversed.

The Office is correct that Veit et al. do not use a glucoamylase having at least 98-99% sequence identity to SEQ ID NO: 2, wherein the sugar concentration is kept at a level below 3 wt % nor a hybrid alpha-amylase comprising an *Aspergillus niger* acid alpha-amylase catalytic domain (CD) and a carbohydrate-binding module (CBM) from *Aspergillus kawachi* alpha-amylase.

However, another significant difference is that Veit et al. disclose a conventional process for producing ethanol, *i.e.*, liquefaction of starch at a temperature which results in gelatinization of the starch, saccharification, and fermentation; and do not teach or suggest a process for producing ethanol by saccharification and fermentation at a temperature between 28°C and 36°C, which is below the gelatinization of starch.

Svendsen et al. disclose a method of constructing variants of a Termamyl®-like alpha-amylase, which is a group of bacterial alpha-amylases.

However, Svendsen et al. do not teach or suggest a hybrid alpha-amylase comprising an *Aspergillus niger* acid alpha-amylase catalytic domain (CD) and a carbohydrate-binding module (CBM) from *Aspergillus kawachi* alpha-amylase.

Moreover, Svendsen et al. also do not teach or suggest a process for producing ethanol by saccharification and fermentation at a temperature between 28°C and 36°C, which is below the gelatinization of starch.

Nagasaka et al. disclose saccharification of raw starch with a *Corticium rolfsii* glucoamylase.

Nagasaka et al. do not teach or suggest saccharification of raw starch with a glucoamylase with at least 98% sequence identity with the sequence of amino acids 1-561 of SEQ ID NO: 2 and an acid alpha-amylase hybrid comprising an *Aspergillus niger* acid alpha-amylase catalytic domain and a CBM from an *Aspergillus kawachii* alpha-amylase.

Moreover, Example 2 of the instant application describes saccharification of milled corn with the following enzyme compositions:

1. *Athelia rolfsii* glucoamylase comprising the sequence of amino acids 1-561 of SEQ ID NO: 2;
2. *Athelia rolfsii* glucoamylase comprising the sequence of amino acids 1-561 of SEQ ID NO: 2 and an acid alpha-amylase hybrid comprising *Aspergillus niger* acid alpha-amylase catalytic domain with *Aspergillus kawachii* alpha-amylase linker and CBM;
3. *Aspergillus niger* glucoamylase; and
4. *Aspergillus niger* glucoamylase and an acid alpha-amylase hybrid comprising *Aspergillus niger* acid alpha-amylase catalytic domain with *Aspergillus kawachii* alpha-amylase linker and CBM.

The glucose concentrations obtained in the saccharification are shown below:

| Treatment | 4 hour Glucose (g/L) |
|--|----------------------|
| 0.263 mg/g DS <i>Aspergillus niger</i> glucoamylase | 33.2 |
| 0.263 mg/g DS <i>Aspergillus niger</i> glucoamylase + 0.034 mg/g DS <i>Aspergillus niger</i> acid alpha-amylase with <i>Aspergillus kawachii</i> alpha-amylase linker and CBM | 40.4 |
| 0.263 mg/g DS <i>Athelia rolfsii</i> glucoamylase | 45.6 |
| 0.263 mg/g DS <i>Athelia rolfsii</i> glucoamylase + 0.034 mg/g DS <i>Aspergillus niger</i> acid alpha-amylase with <i>Aspergillus kawachii</i> alpha-amylase linker and CBM | 63.2 |

As shown in the table and as explained in the prior response, the addition of the acid alpha-amylase hybrid comprising *Aspergillus niger* acid alpha-amylase catalytic domain with *Aspergillus kawachii* alpha-amylase linker and CBM to *Aspergillus niger* glucoamylase resulted in an increase of the glucose concentration from 33.2 to 40.4 g/l, or about 21.7% $((40.4 - 33.2)/33.2)$, whereas the addition of the acid alpha-amylase hybrid comprising *Aspergillus niger* acid alpha-amylase catalytic domain with *Aspergillus kawachii* alpha-amylase linker and CBM to the *Aspergillus niger* glucoamylase resulted in an increase of the glucose concentration from 45.6 to 63.2 g/l, or about 38.6% $((63.2 - 45.6)/45.6)$, a significantly greater increase. Since these results are not predicted by the prior art, they are surprising and unexpected.

Moreover, persons of ordinary skill in the art would expect that the use of a glucoamylase with a high sequence identity with the sequence of amino acids 1-561 of SEQ ID NO: 2 and an acid alpha-amylase hybrid comprising an *Aspergillus niger* acid alpha-amylase catalytic domain and a CBM from an *Aspergillus kawachii* alpha-amylase would achieve similar results.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

All required fees were charged to Novozymes North America, Inc.'s Deposit Account No. 50-1701 at the time of electronic filing. The USPTO is authorized to charge this Deposit Account should any additional fees be due.

Respectfully submitted,

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